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INVESTIGATIONS ON THE INFLUENCE OF INSULIN AND ADRENALIN IN RABBITS WITH ALIMENTARY FATTY LIVER

THE EFFECT OF INSULIN AND ADRENALIN ON THE CONTENT OF FAT AND GLYCOGEN IN LIVER AND MUSCLES AND ON THE CONTENT OF FAT AND SUGAR IN BLOOD

BY

SVEIN LUNDE SVEINSSON

WITH 26 FIGURES IN THE TEXT AND 2 PLATES

SKRIFTER UTGITT AV DET NORSKE VIDENSKAPS-AKADEMI I OSLO
I. MAT.-NATURV. KLASSE. 1939. No. 8

På grunnlag av nærværende avhandling
er forfatteren kreert til doctor

medicinae

ved ~~DET KONGELIGE FREDERIKS~~
UNIVERSITET I OSLO
13. april 1940.

OSLO
I KOMMISJON HOS JACOB DYBWAD
1939

The content of fatty acids in the muscles shows only insignificant changes in the course of 4 of the 5 control tests.

In an experiment in which the respiratory metabolism was investigated there was observed an increase in the output of carbon dioxide and a somewhat smaller increase in the oxygen intake. These phenomena are assumed to be due to a slight effusion of adrenalin during the experiment.

VI. Experiments with Insulin.

On rabbits with fatty livers a number of experiments have been carried out in order to study the effect of administration of insulin on the content of fat and glycogen in the liver. The liver's content of fatty acids and glycogen was determined at intervals of a few hours, in most cases three times altogether during an experiment. At the same time were determined the changes which occurred in the content of fatty acids and glycogen in certain muscles and in the blood's content of fat and sugar. The insulin employed was the ordinary commercial preparation manufactured by the Apotekernes Laboratorium, Oslo (Insulin A. L.).[1]

1. Insulin Dosage.

In several of the experiments the insulin was injected intramuscularly, but otherwise it was dissolved in a 0.9 per cent NaCl-solution and administered slowly by intravenous infusion. By the latter mode of procedure a rapid effect from the insulin is attained and by regulating the rate of inflow a steady administration of the hormone can be secured. The quantities of insulin administered varied somewhat, but in all experiments the doses were rather large. In the 8 experiments where insulin was given intravenously the dose varied from 14.5 to 34 I. U. The average rate of infusion ranged from about 3 to about 10 I. U. per hour. The object in using slow intravenous infusion of these large doses of insulin was to secure a prolonged effect of the hormone and to render this effect as intense as possible in order thereby to counterbalance the drawback attaching to these experiments, namely, that owing to the special mode of arrangement they cannot be of very long duration.

The use of large doses of insulin without simultaneous administration of carbohydrates is inadmissible in the case of such animals as rabbits, so long as they are in waking state, since they will then very soon fall into hypoglycæmic coma and perish in the attendant convulsions. During narcosis, however, large doses of insulin may be injected, which in several of the experiments cause the blood sugar to sink to very low values, probably

[1] This preparation contains 40 I. U. insulin per 1 ml and is acidified by HCl to pH = 3. The insulin A. L. is at all times controlled at the University physiological institute by Dr. O. J. Strand.

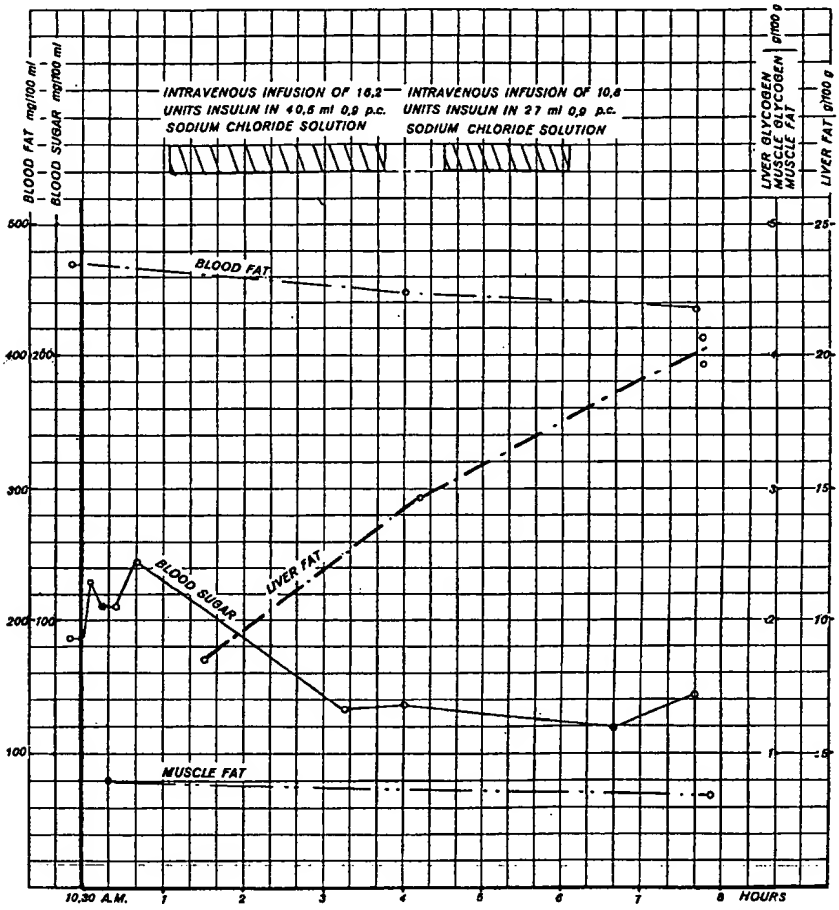

Fig. 13. Experiment No. 22. Intravenous infusion of insulin in 0.9 per cent NaCl-solution.

right down to values corresponding to the residual reduction in the blood, without leading to the death of the animals. There is therefore every reason to suppose that it is owing to the non-occurrence of convulsions during anesthesia that the animals are able to stand the large doses of insulin so long as they are in narcotised state. On the other hand, it is also very often seen that even considerable quantities of insulin fail to have so much effect in reducing the content of blood sugar as might be expected.

2. Experimental Results.

The results attained in the insulin experiments are assembled in tables (14 a—d) in the same manner as was done with the control experiments. Two of the experiments are moreover illustrated graphically in Figs. 13 and 14.

Table 14 a.

No.	Blood sugar: Fasting blood sugar, mg/100 ml	Blood sugar: Expressed in p. c. of the fasting blood sugar is the: Increment in blood sugar after preparation for sample taking	Increment in blood sugar after sample taking and preparation therefor	Maximum fall in blood sugar	Blood fat: Fasting blood fat, mg/100 ml	Blood fat at close of the experiment, mg/100 ml	Change in blood fat in p. c. of fasting value	Urine: Quant. in ml.	N in mg	Liver: Weight in g
16	96	31	62	22	612	554	− 9.5	-	-	70
17	106	22	32	54	480	459	− 4.4	-	-	90
18	105	6	22	45	593	508	− 14.3	-	-	87
19	105	10	18	39	595	534	− 10.2	1	-	80
20	96	-	-	54	535	-	-	-	-	69
21	102	-	-	75	458	419	− 8.5	-	-	59
22	95	14	32	37	471	435	− 7.6	-	-	101
23	122	6	33	75	510	449	− 12.0	12	-	102
24	95	0	− 4	77	524	443	− 15.5	40	-	98
25	104	-	-	78	485	-	-	5	64	66
26	110	-	-	66	-	-	-	-	-	70
27	114	-	-	60	376	369	+ 5.3	8	123	-
28	80	-	133	39	400	430	+ 7.5	-	-	106
29	111	37	51	69	504	377	− 25.2	18	262	105

Table 14 b.

No.	Liver glycogen: In percentage of wet weight of liver: 1st sample	2nd sample	3rd sample	Change in liver glycogen between first and last sample reckoned per hour: In p. c. of initial value	In p. c. of liver weight	In mg per kg body weight	Liver fat: In percentage of wet weight of liver: 1st sample	2nd sample	3rd sample	Change in liver fat between first and last sample reckoned per hour: In p. c. of initial value	In p. c. of liver weigh
16	0.40	0.28	0.26 0.24	− 6.1	−0.02	− 6	28.60	29.20	25.75 27.80	− 1.0	−0.30
17	0.65	0.30	0.35	− 7.3	−0.05	− 19	27.65	28.90	27.75	+ 0.1	−0.02
18	0.48	0.25	0.14	− 11.0	−0.05	− 25	21.71	19.84	23.50 18.85	− 0.4	−0.08
19	0.51	0.19	0.14 0.14	− 9.3	−0.05	− 16	26.00	17.65	23.10 17.90	− 2.6	−0.69
20	4.11	3.44	0.12 1.39	− 11.2	−0.46	−205	10.15	11.94	16.48	+ 10.3	+ 1.04
21	3.23	2.13	1.32 1.33	− 12.1	−0.39	−151	9.93	11.70	16.70 18.60	+ 15.3	+ 1.5
22	-	-	1.17 -	-	-	-	8.60	14.78	20.63 19.60	+ 18.6	+ 1.6
23	2.95	2.34	1.67	− 6.4	−0.19	− 86	20.43	20.80	lost	-	-
24	4.20	3.00	1.47 2.67	− 4.7	−0.20	− 84	18.75	19.48	26.72 25.96	+ 5.6	+ 1.0
25	0.95	1.07	2.86 0.69	− 4.3	−0.04	− 12	14.45 12.60	16.75	17.15	+ 4.1	+ 0.5
26	3.80	3.23	0.69 2.25	− 6.8	−0.26	-	17.08 16.55	16.65	18.58 23.10	+ 4.0	+ 0.6
27	3.77	1.31	2.19 -	− 9.9	−0.34	-	17.13 17.52	19.94	-	+ 2.4	+ 0.
28	3.19 3.39	1.30 2.85	0.56	− 11.4	−0.39	− 145	2.38 2.80	2.89	2.90	+ 1.7	+ 0.
29	3.80	3.07	0.63 1.85 2.08	− 6.4	−0.24	− 81	2.37 2.58	2.69	2.39	− 0.5	− 0.

Table 14c.

No.	Muscle glycogen: In per cent of muscles' wet weight, 1st sample	Muscle glycogen: In per cent of muscles' wet weight, 2nd sample	Muscle glycogen: Change in muscle glycogen between first and last sample, In p. c. of initial value	Muscle glycogen: Change in muscle glycogen between first and last sample, In p. c. of initial value per hour	Muscle fat: In per cent of muscles' wet weight, 1st sample	Muscle fat: In per cent of muscles' wet weight, 2nd sample	Muscle fat: Change in muscle fat between first and last sample, In p. c. of initial value	Muscle fat: Change in muscle fat between first and last sample, In p. c. of initial value per hour	Liver: Weight in g
16	*0.58*	*0.41*	*−17.4*	*−2.7*	*0.88*	*0.63*	*−28.4*	*−4.3*	*70*
	0.51	*0.49*							*90*
17	*0.49*	*0.49*	*− 8.1*	*−1.2*	*0.83*	*0.75*	*− 9.6*	*−1.4*	*87*
	0.49	*0.41*							*80*
18	*0.46*	*0.51*	*+12.4*	*+1.8*	*0.81*	*0.84*	*+ 3.7*	*+0.5*	*69*
	0.43	*0.49*							*59*
19	*0.60*	*0.32*	*−46.0*	*−5.4*	*0.79*	*0.80*	*+ 1.3*	*+0.1*	*101*
	0.62	*0.34*							*102*
20	0.63	0.54	− 17.0	−2.5	0.94	0.54	−43.0	−6.2	98
	0.66	0.53							66
21	0.53	0.61	+ 6.5	+0.8	1.00	0.70	−30.0	−3.8	70
	0.54	0.53							-
22	-	-	-	-	0.81	0.69	−15.0	−2.0	*106*
23	0.46	0.33	−25.8	−3.3	0.68	0.51	−25.0	−3.2	*105*
	0.43	0.33							
24	0.71	0.47	−27.7	−3.5	0.73	0.45	−38.0	−4.8	
	0.66	0.52							
25	0.52	0.41	−18.2	−1.8	0.96	0.86	−10.4	−1.0	
	0.47	0.40							
26	0.35	0.45	+46.2	+7.2	0.70	0.63	−10.0	−1.6	
	0.30	0.50							
27	0.62	0.59	− 1.6	−0.3	1.03	1.14	+10.7	+1 6	
		0.63							
28	*0.67*	*0.39*	*−41.8*	*−5.4*	*0.89*	*0.75*	*−15.7*	*−2.0*	
29	*0.51*	*0.42*	*−17.7*	*−2.2*	*0.74*	*0.61*	*−17.6*	*−2.2*	

e in liver fat
i first and last
le reckoned
er hour

of lue	In p. c. of liver weight
›	*−0.30*
r	*−0.02*
f	*−0.08*
5	*−0.69*
3	*+1.04*
3	*+1.52*
5	*+1.60*
	-
6	*+1.05*
1	*+0.57*
o	*+0.66*
4	*+0.41*
7	*+0.04*
5	*−0.01*

Table 14d. Summary of Tables 14a—14c.

No.	Date	Weight of animal in g	Maximum fall in blood sugar from initial value in p. c. thereof	Change in blood fat during experiment in p. c. of initial value	Change per hour (in p. c. of liver weight) of liver's content of: glycogen	Change per hour (in p. c. of liver weight) of liver's content of: fatty acids	Change per hour (in p. c. of initial value) of muscles' content of: glycogen	Change per hour (in p. c. of initial value) of muscles' content of: fatty acids	Remarks
16	*9/6-36*	*2640*	*−22*	*− 9.5*	*−0.02*	*−0.30*	*−2.7*	*−4.3*	*Subcut. injec. of 2 I. U. insulin × 2.*
17	*11/6-36*	*2250*	*−54*	*− 4.4*	*−0.05*	*+0.02*	*−1.2*	*−1.4*	*Subcut. injec. of 4 I. U. insulin × 2.*
18	*15/6-36*	*2040*	*−45*	*−14.3*	*−0.05*	*−0.08*	*+1.8*	*+0.5*	*Subcut. injec. of 4 I. U. insulin × 2.*
19	*18/6-36*	*2350*	*−39*	*−10.2*	*−0.05*	*−0.69*	*−5.4*	*+0.1*	*Subcut. injec. of 6 I. U. insulin × 2.*
20	11/8-36	1530	−54	-	−0.46	+1.04	−2.5	−6.2	I. ven. inf. of 14.5 I. U. insulin in 36 ml 0.9 p. c. NaCl-sol.
21	20/8-36	1520	−75	− 8.5	−0.39	+1.52	+0.8	−3.8	I. ven. inf. of 18.7 I. U. insulin in 46 ml 0.9 p. c. NaCl-sol.
22	24/8-36	1750	−37	− 7.6	-	+1.60	-	−2.0	I. ven. inf. of 27 I. U. insulin in 67.6 ml 0.9 p. c. NaCl-sol.
23	27/8-36	2240	−75	−12.0	−0.19	-	−3.3	−3.2	I. ven. inf. of 22.4 I. U. insulin in 42 ml 0.9 p. c. NaCl-sol.
24	31/8-36	2320	−77	−15.5	−0.20	+1.05	−3.5	−4.8	I. ven. inf. of 31.4 I. U. insulin in 78.5 ml 0.9 p. c. NaCl-sol.
25	14/1-37	2200	−78	-	−0.04	+0.57	−1.8	−1.0	I. ven inf. of 34 I. U. insulin in 70 ml 0.9 p. c. NaCl-sol.
26	5/8-37	-	−66	-	−0.26	+0.66	+7.2	−1.6	I. ven. inf. of 21.6 I. U. insulin in 49 ml 0.9 p. c. NaCl-sol.
27	12/8-37	2310	−60	+ 5.3	−0.34	+0.41	−0.3	+1.6	I. ven. inf. of 31.4 I. U. insulin in 60.4 ml 0.9 p. c. NaCl-sol.
28	*20/11-36*	*2820*	*−39*	*+ 7.5*	*−0.39*	*+0.04*	*−5.4*	*−2.0*	*I. ven. inf. of 56.3 I. U. insulin in 70.4 ml 0.9 p. c. NaCl-sol.*
29	*23/11-36*	*3150*	*−69*	*−25.2*	*−0.24*	*−0.01*	*−2.2*	*−2.2*	*I. ven. inf. of 39.6 I. U. insulin in 45 ml 0.9 p. c. NaCl-sol.*

Rabbits Nos. 16—19 fed on 40 p. c. fat diet.
„ „ 20—27 „ „ 20 „ „
„ „ 28 and 29 fed on normal diet.

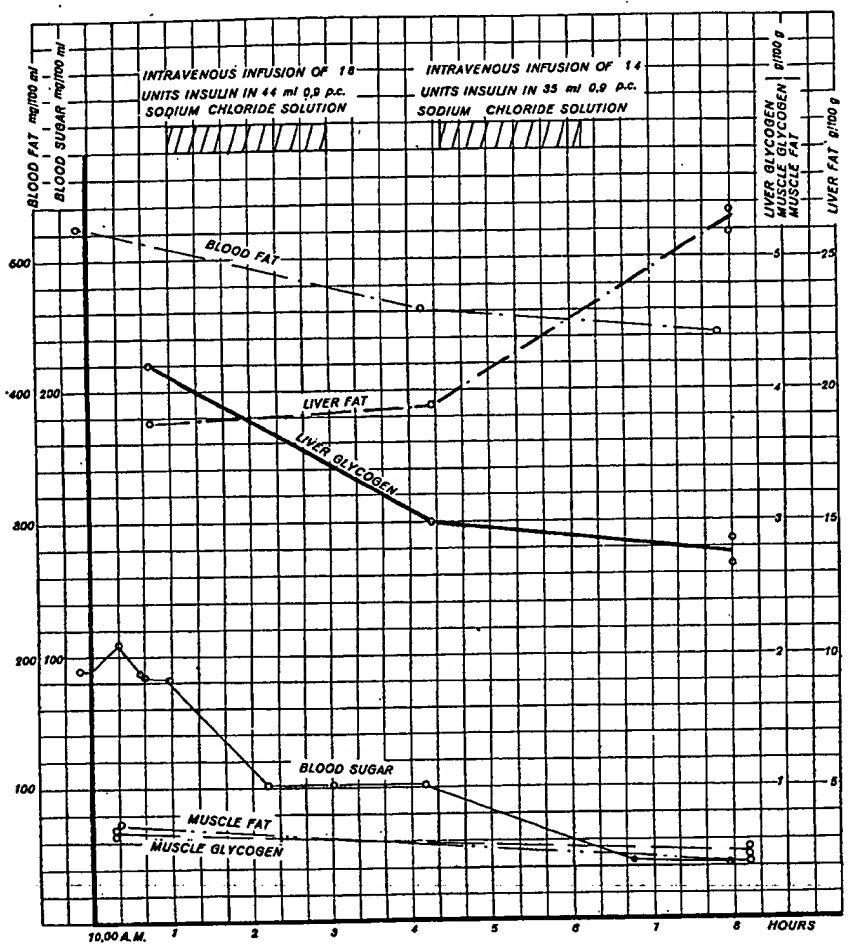

Fig. 14. Experiment No. 24. Intravenous infusion of insulin in 0.9 per cent NaCl-solution.

a. *Blood Sugar and Blood Fat.*

The blood sugar is found to decrease as a result of the administration of insulin, but this decrease is, as already mentioned, not so great in all the experiments as might be expected. In Experiment No. 16 the blood sugar declines after injection of 2 I. U. subcutaneously from 150 mg/100 ml to 78 mg/100 ml after the lapse of 2½ hours and a renewed subcutaneous injection of insulin does not effect any further reduction of the blood sugar. In Experiment No. 19 a subcutaneous injection of 6 I. U. leads to a fall in blood sugar from 123 mg per cent to 68 mg per cent in the course of 3 hours and a further injection of 6 I. U. subcutaneously fails to lower the blood sugar more than to 59 mg per cent. In Experiment No. 22 an intravenous injection of 16.2 I. U. in 40.5 ml. of 0.9 per cent NaCl-solution occasions a fall of blood sugar from 123 mg per cent to 65 mg per cent in the course of about 3 hours and a continued infusion of 10.8 I. U. in saline solution brings about no further decrease of blood sugar. One international

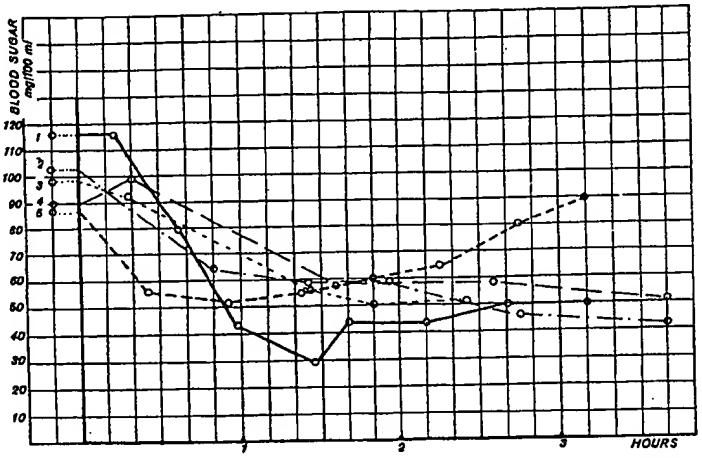

Fig. 15. Blood sugar curves after administration of insulin at the point of time 0.
1. Rabbit, 1.8 kg, with fatty liver, in waking state. Subcutaneous injection of 1.6 I. U.
2. Rabbit, 2.1 kg, with normal liver in narcosis. Intravenous injection of 5 I. U.
3. Rabbit, 2.1 kg, » » » » » Intramuscular injection of 10 I. U.
4. Rabbit, 2.0 kg, » » » » » { Intravenous injection of 10 I. U. / Intramuscular injection of 10 I. U.
5. Rabbit, 2.3 kg, » » » » » Intravenous injection of 2 I. U.

insulin unit equals about ⅓ of the original Toronto unit. According to the given definition a subcutaneous injection of 1 Toronto unit into a fasting rabbit weighing about 2 kg shall in the course of 3 or 4 hours bring the blood sugar down to the cramp-producing threshold value of 45 mg per cent. Even if we reckon with a considerable individual variation in the extent of this fall in blood sugar, yet we will come to the result that with the dosage employed there is not always attained the same blood-sugar-reducing effect on rabbits in Pernocton-narcosis as with a smaller dose of insulin on animals in waking state. That the weakened effect of insulin on the blood sugar in these animals is not due to their having been kept on a high fat diet is proved by the following experiment (Fig. 15): A rabbit which had been kept on a high fat diet for 15 days and had afterwards fasted for 23 hours is given 1.6 I. U. of insulin in 2 ml aq. dest. subcutaneously in waking state and the blood sugar curve is then recorded. The appearance of the curve accords with what is found in normally fed rabbits after a fast of 24 hours. On the other hand, the effect of insulin during narcosis has been studied in normally fed rabbits. In 4 experiments different doses of insulin dissolved in 1 ml aq. dest. were given, sometimes intravenously, sometimes intramuscularly. It appears from these experiments that large doses of insulin in narcosis do not occasion any greater fall in the blood sugar than does a small dose (2 I. U.). Whether a small dose of insulin has less effect when given during narcosis than it has in waking state cannot be seen from these few experiments. To decide that question it would be necessary to have a large body of material for investigation. MULINOS (104) found that insulin in small doses acted in

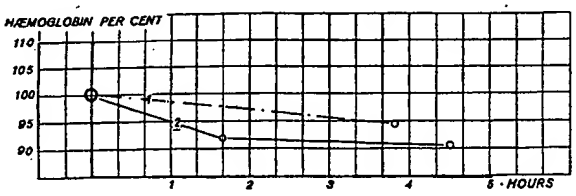

Fig. 16. Hæmoglobin content in blood in two rabbits during infusion of insulin dissolved in 0.9 per cent NaCl-solution. Sahli's method.
1. Infusion of 21.5 I. U. in 49 ml saline solution.
2. » » 31.4 » » 60 » » »
Hæmoglobin content before infusion begins = 100 per cent.

the same manner during Pernocton-narcosis as on an animal in waking state. The fact that in several of the experiments in the present work a maximum fall in blood sugar was not attained after large doses of insulin may be due to the method of administration employed, namely, slow intravenous infusion, but it is also possible to maintain that the effusion of adrenalin, which may be presumed to take place during these experiments, is the cause of the restriction in fall of blood sugar after insulin that is here observed.

It has already been mentioned that if the insulin is given to the animals twice during the experiment the effect of the last dose will in most cases be weaker than that of the first. In several of the experiments the second dose merely contributes to keep the blood sugar at the level to which it had been brought down by the first dose, even if that level was not particularly low.

In order to be able to judge correctly respecting the variations in blood sugar under the influence of insulin when considerable quantities of liquid are infused at the same time it is necessary also to have some knowledge as to the organism's water economy during the experiments. If changes take place in the blood during the experiment, either in the form of thickening or dilution, they will lead to variations in the blood's percental content of sugar and must consequently be taken into account when it is desired to study the actual effect of the insulin on a particular one of the components of the blood. When dealing with the control experiments it was mentioned that intravenous injection of salt water brings about a dilution of the blood. The question now is whether the addition of insulin to the liquid injected will occasion an alteration in the effect produced by the saline solution. The action of insulin on the content of water in the blood and on the excretion of urine has been previously studied by several investigators. HALDANE, KAY and SMITH (68) gave large doses of insulin to non-narcotised rabbits and found that the blood increased in volume, which they deduce from the fact that the figures for hæmoglobin, hæmatocrite values and number of red blood-corpuscles declined. VOLLMER and SEREBRIJSKI (138) after making some Vollhard water-tests on human subjects found an anti-diuretic action of insulin. COLLAZO and DOBREFF (29) studied the urine

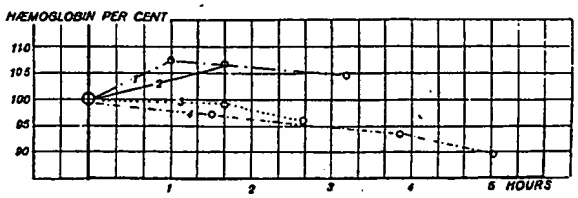

Fig. 17. Hæmoglobin content in blood in 4 rabbits after injections of insulin at the point of time 0.

1. Intravenous injection of 1 I. U.
2. { Intravenous injection of 10 I. U.
   { Intramuscular injection of 10 I. U.
3. Intravenous injection of 5 I. U.
4. Intramuscular injection of 10 I. U.

Hæmoglobin content before infusion begins = 100 per cent.

excretion in dogs after insulin. From the tables published by these authors it appears that the excretion of urine decreases in the first 1½ to 3 hours after injection of insulin, whereupon the quantity of urine increases. As the insulin was given intravenously and consequently exercised its effects immediately, the conclusion to be drawn from the experiments must be that insulin reduces the excretion of urine. SIEBECK (126) maintains that the influence of insulin on the water economy of the organism seems to vary and he points out that edema may occasionally develop during insulin treatment. STAUB (129) sums up the results arrived at by the different investigators respecting the action of insulin on the organism's water economy as showing that small doses of insulin which do not occasion any great changes in the blood sugar likewise fail to produce any material alterations in the water content of blood and tissue in persons and animals with normal metabolism. Large doses, however, lead to retention of water and salt in the tissues.

In two experiments, where insulin in 0.9 per cent NaCl-solution was injected into fat-fed rabbits, the present author investigated the hæmoglobin content of the blood by Sahli's method (Fig. 16). The results show that the amount of hæmoglobin in the blood here decreases, just as in the experiments where salt water alone was injected. The quantities of insulin injected in these experiments accordingly does not seem to affect the hæmoglobin concentration in the blood. In some experiments on normally fed rabbits (in narcosis and water-bath) — Fig. 17 — in which the insulin was dissolved in a very small quantity of liquid (1 ml) and injected intravenously or intramuscularly, the hæmoglobin content of the blood (estimated colorimetrically as CO-hæmoglobin) was found in some cases to have increased, in others to have declined. From Table 14 a it is seen, however, that insulin exercises a considerable influence on the organism's water economy. In spite of the large quantities of water injected together with the insulin into the fat-fed rabbits the amount of urine excreted during the

period of experiment is very scanty. In 3 of the insulin experiments during which from 42 to 70 ml of liquid were injected the quantity of urine excreted ranges from 5 to 12 ml, and the urine is concentrated and slightly viscid. In one of the experiments, however, where 78.5 ml of physiological saline solution were injected, the urine excretion amounted to 40 ml. These findings show that a considerable portion of the liquid injected is retained in the organism, but at the same time they show that the cause of this retention of water is hardly to be sought for in impaired renal function. The fact that the urine excreted is concentrated suggests that the cause of the reduced urine excretion must be looked for extrarenally, and then most probably in a retention of water in the tissues.

The moderate degree of blood dilution occurring during the insulin infusions occasions a slight decrease in concentration of the different constituents of the blood, corresponding to the percental fall in the Sahli values.

In all but one of the insulin experiments a reduction of the blood's content of fat is seen to take place. The extent of this decrease varies from 4 to 16 per cent of the initial value. This decline in the percentage of fat contained in the blood in the separate experiments will have been occasioned, at least in part, by the dilution of the blood observed during the injection of insulin. That part of the percental decrease in the fat content of the blood which is due to this dilution must be deducted from the figures given in the table in order to ascertain what percentage of the fall in blood fat is a direct consequence of the action of the insulin. Determination of the blood dilution that occurs has not been made in all the insulin experiments and for that reason it is difficult to draw more definite conclusions as to the direct action of insulin on the blood fat. The circumstance that the blood fat also decreases during those insulin experiments where no intravenous infusion of liquid is made, but where the insulin is injected intramuscularly in concentrated solution, seems, however, to indicate that in the experiments performed there is a tendency to a fall in the fat content of the blood under the influence of insulin.

b. *Liver Glycogen and Liver Fat.*

The liver's content of glycogen and fat has been investigated in the same manner as in the control tests, the results being shown in the tables. The glycogen content in the liver decreases during the experiments, but in greatly varying degree, as was also found in the control experiments. The changes which the glycogen in the liver undergoes, expressed in percentage of the content at the beginning of the experiment and calculated per hour, range from 4,3 to 12,1 per cent, as against 1,8 to 12 per cent in the control experiments, and the loss of glycogen, expressed in mg reckoned on the basis of the liver-weight at the conclusion of the experiment, varies from 6 to 205 mg per kg of body-weight per hour, as against a corresponding variation

of from 8 to 178 mg per kg in the control experiments. In spite of administration of insulin in large doses there is thus found no distinct alteration in the rapidity with which the glycogen in the liver decreases in a fasting rabbit with fatty liver. There has not been detected any increase in the loss of glycogen from the liver which would indicate an increased break-down of liver glycogen. Neither has there occurred any retardation of the loss of glycogen. Especially it must be pointed out that in these experiments with administration of insulin there has in no case occurred any increase of the glycogen content in the liver which might point either to a conveyance of carbohydrates from stores elsewhere in the organism or to new-formation of carbohydrates.

The content of fatty acids in the liver in the 4 experiments where the insulin is given to the animals subcutaneously shows no alterations of such extent that they can be assigned any importance. These four rabbits had been fed on a diet containing 40 per cent of coco-fat and the fatty acid content of the liver was in all of them very high. In the 8 experiments on rabbits which had received a 20 per cent fat diet there occur, however, during intravenous administration of insulin considerable changes in the percentage of fatty acids contained in the liver. In all experiments the content of fatty acids in the liver increases and in some cases this increase is very considerable. Expressed in percentage of the content at the beginning of the experiment and reckoned per hour, this increase varies from 2,4 up to as much as 18.6 per cent. The increase is greatest in Experiments 20, 21 and 22, the percentage of fatty acids in the liver having risen from 10.15 to 16.48 in No. 20, from 9.93 to 17.65 in No. 21 and from 8.60 to 20.12 in No. 22. But also in other experiments the percentage of fatty acids has risen considerably, in No. 26, for instance, from 16.82 to 20.84, in No. 25, from 13.53 to 17.15 and in No. 27, from 17.33 to 19.94 in the course of 6 hours. In one single experiment no increase of the fatty acid content in the liver was observed during infusion of insulin, but this experiment is not included in the table, as there may be some doubt as to its validity, seeing that the liver in this case showed pathological changes, being pervaded by numerous cystic formations which were assumed to originate from infection by intestinal worms.

c. *Muscle Glycogen and Muscle Fat.*

The glycogen content of the muscles shows a somewhat varying behaviour during administration of insulin. In most experiments the quantity of glycogen in the symmetrical muscles examined decreases, in some cases very considerably, while in some few experiments an increase of muscle glycogen occurs. In one of these latter the increase is very great. In this experiment, however, the percentage of glycogen in the two pairs

of muscles examined was at the outset very low (0.33 per cent). It must be remembered that also in the control tests considerable variations in the content of muscle glycogen might be observed, and it cannot be concluded from the experiments with insulin that the variations here seen are greater than those found in the control experiments. In order to ascertain whether the content of water in the muscles undergoes any change during the administration of insulin examination of the quantity of water in symmetrical muscles was made in two of the experiments. As is seen from Table 15, the content of water in the muscles remains almost unchanged during these experiments.

The fat content in the muscles decreases under the influence of insulin in most of the experiments, but in very different degree. In some cases the loss of fat from the muscles is very great and in one experiment it amounts to as much as 43 per cent of the initial content. In another case the decrease during the whole period of experiment is 38 per cent. No movement of the muscles takes place during the time the experiment lasts and the most reasonable explanation of the decreasing fat content therefore is that the fat must in some form or other be transported away from the muscle through the action of the insulin. As to why this transference of muscle fat is greater in some experiments than in others no plausible explanation can be given on the basis of the analyses performed.

d. *Summary of Results.*

The results of the investigations respecting the effects produced by administration of large doses of insulin on fat-fed rabbits may be summed up as follows:

Whereas the glycogen in the liver decreases in almost the same manner as in the control experiments, the fatty acids in that organ show a considerable, though somewhat varying, increase under the influence of insulin in most of the experiments. As regards the muscles the findings show some variation. In most cases, however, there is a loss both of glycogen and fatty acids.

The blood sugar declines during dosage with insulin, but in several of the experiments the fall is not of maximum extent, in spite of the employment of large doses of insulin. The blood fat also decreases somewhat during the experiments. This action of insulin on blood sugar and blood fat is partly of secondary nature, seeing that the intravenous infusion of liquid leads to dilution of the blood, and as far as regards the blood fat, the decrease of which is small, the dilution of the blood affords a certain amount of explanation. Meanwhile there are also signs to indicate that the insulin directly reduces the fat content of the blood.

3. Discussion.

a. *The Action of Insulin on the Fat Content of Liver and Muscles.*

The most prominent feature in this picture of the effects of insulin on rabbits that were fed on high fat diet is the great accumulation of fatty acids which takes place in the liver. In what manner shall we now explain this great increase in the percentage of fatty acids in this organ? As already mentioned when speaking of the control experiments, a reduction of the content of water in the liver will naturally bring about some increase of the percental proportion of the various solid constituents. A drying up of the liver would lead to an increased percentage of fatty acids therein. But no such pronounced desiccation and skrinkage of the liver as would afford a reasonable explanation of the findings made have been observed. Nor would it be reasonable to expect such an effect in these experiments. The tendency of the insulin is, as we know, to bring about a retention of water in the tissues rather than a desiccation. In two of the experiments the content of water in the liver was determined at the beginning and at the close of the experiment without any material alterations being demonstrated (Table 15). Neither is it reasonable to suppose that a change in the blood content of the samples taken in these experiments could explain the great alterations in the percentage of fat in the liver. Moreover, in several of the experiments the quantities of fat accumulated are so large that they cannot be explained by the assumption that the glycogen in the liver has been converted into fat through the action of the insulin. Neither is the loss of sugar and glycogen observed in blood and muscle under the influence of insulin sufficient alone to explain the accumulation of the large quantities of fat in the liver. It then remains for us to assume that the fat which

Table 15.

Content of Water in Liver and Muscles during Administration of Insulin.

Experiment No.	Content of water in 2 pairs of symmetrical muscles in % of wet weight			Content of water in liver in % of wet weight	
	Muscle	At beginning of experiment	At end of experiment	At beginning of experiment	At end of experiment
26	M. tib. ant.	79.2	79.0	Sample 1 (Wt. 17.8 mg) 60.4	Sample 1 (Wt. 28.4 mg) 62.3
	M. ext. dig.	80.5	78.5	Sample 2 (Wt. 70.0 mg) 64.0	Sample 2 (Wt. 20.5 mg) 59.0
27	M. tib. ant.	76.4	76.3	Sample 1 (Wt. 63.9 mg) 57.0	Sample 1 (Wt. 67.1 mg) 57.9
	M. ext. dig.	76.1	75.8	Sample 2 (Wt. 32.6 mg) 56.2	Sample 2 (Wt. 101.3 mg) 55.0

accumulates in the liver has, at any rate in part, been conveyed thereto from other parts of the organism.

It is of especial interest to note that this effect on the liver from intravenous administration of insulin is either not at all or only in slight degree apparent in rabbits which have been receiving ordinary food and have a normal content of fat in the liver. In Experiments Nos. 28 and 29 very small variations occur in the fat content of the liver during administration of large doses of insulin. It seems difficult to give an explanation of this difference between normally fed and fat-fed rabbits, but it is natural to suppose that the difference must be regarded in connection with the diversity existing in intermediary metabolism between rabbits receiving normal food and those on high fat diet.

The effect of insulin on the different forms of fatty liver have earlier been made the subject for a number of investigations. Already in 1922 BANTING and co-workers (5) was able to state that administration of insulin to pancreas-diabetic dogs caused an improvement in the fatty liver in these animals and reduced the fat content of the blood. While non-treated pancreas-diabetic dogs had a fat content in the liver of from 9.9 to 14.1 per cent, similar animals after treatment with insulin had not more than from 2.2 to 7.4 per cent of fat, and the blood fat fell from 1 per cent to between 0.3 and 0.5 per cent. MACLEOD (95) reports that the fat content in the liver of pancreas-diabetic dogs already a couple of days after they had been given glucose and insulin lay considerably lower than in non-treated animals. HOTTA (70) found in the liver of 2 pancreas-diabetic dogs an average fat content of 18.5 per cent. Three such dogs which had been treated with large doses of insulin showed, on the other hand, an average fat content of 5.03 per cent in the liver. He found no distinct effect from the insulin on the fat content in the muscles. WERTHEIMER (140—141) found in his phlorizin-diabetic dogs that fatty liver could be prevented from developing if insulin was given together with the phlorizin. He was likewise able by administration of insulin to obtain improvement in phlorizin fatty liver that had already developed. Also MÖBIUS (105) found the fatty liver less pronounced in dogs after phlorizin when insulin was administered at the same time. The content of fat in the muscles, however, was higher in the animals which had been given insulin together with the phlorizin. ARNDT and GREILING (2) on the other hand found in rabbits which had been given phosphorus and insulin together that the liver contained increased amounts of fat but no glycogen. RAPER and SMITH (118) studied the effects of insulin on the fat content in liver and muscles in cats, in some of which a high percentage of fat (up to 32 per cent) had been obtained in the liver by fat-feeding. The experiments were carried out on decerebrated cats in which also the hypophysis had been destroyed. Raper and Smith found that, when the action of the insulin was so marked that it led to hypoglycæmia (4.5 to 10 clinical units per kg of body-weight), the content of

fat in the liver decreased. In the muscles, however, the percentage of fat increased, likewise the percentage of fat in the blood.

Also on animals with normal content of fat in the liver several investigations have been carried out respecting the influence of insulin on the quantity of fat in different organs. PAGE, PASTERNAK, and BURT (111) in experiments of short duration on rabbits found no change in fat content of the liver after large doses of insulin, whereas the total-fat and especially the content of phosphatides in serum decreased under the influence of insulin. SATOH (122) observed no decided influence on the fat percentage in the liver of normally fed cats during administration of insulin. THEIS (135), after having given to rabbits on ordinary diet 3 units of insulin daily for 3 weeks, noted a decline in the quantity of fat in the liver. In short experiments, however, where the liver was analysed 6 hours after administration of a single dose of insulin there was a slight increase in the content of fat. In all experiments with insulin the relative percentage of phosphor lipides in the liver fat decreased. DUDLEY and MARRIAN (47) injected insulin into mice and determined the content of fat in the liver after insulin convulsions had set in. They could find no increase of the fat content in the liver during the experiment. SOPP and SELBACH (127) investigated the blood sugar and blood fat in dogs which had first been given food rich in carbohydrates without insulin for 8 days and afterwards the same diet with insulin. The animals were then killed and the content of fat and glycogen in liver and muscles was determined. The percentage of blood fat increased under the influence of insulin, the increase being greatest when the blood sugar content was lowest. The results of the analyses of some organs were compared with findings made in dogs that had been kept on the same high carbohydrate diet without insulin and the investigators then found that under the influence of insulin there had taken place a decrease in the liver's content of glycogen and fat, while the percentage thereof in the muscles had increased. OMURA and NITTA (109) made determination of fat *ad modum* Soxleth in a number of organs in rabbits after injection of insulin and found that the fat content increased in the heart, muscles and kidneys, but showed a tendency to decrease in the liver. In fat determinations made in the whole organism of mice both after insulin alone and after insulin + glucose an increase of the fat content was found in both cases. VENDÉG (137) studied the effect of insulin on dogs which had been kept in Pernocton anesthesia during the experiment. The arrangement of the experiment was similar to that adopted in the present work, samples for fat determination being taken from the liver of the same animal at two different times. The variations in blood fat were likewise examined. The determination of fat in liver and blood was made in large portions of tissue after Soxleth's method. In experiments of from 3 to 6 hours' duration on fasting dogs Vendég found after injection of 1 I. U. per kg of body-weight per 1½ hours that the liver fat increased in 5 out of 6 experiments.

The quantity of fat in the blood showed in general a decreasing tendency. HIMWICH and SPIERS (69) in experiments on dogs found that insulin in doses of from 1 to 2 units per kg which produce distinct hypoglycæmia cause a reduction of the blood fat content. TANGL (133), on the other hand, after administering one large dose of insulin to one single dog, found that the blood fat content increased.

From the findings of these authors it appears that in animals with greatly increased fat content in the liver, due to pancreas diabetes or phlorizin diabetes, administration of insulin will cause the percentage of fat to decrease. In cats in which fatty liver has been produced by feeding on a diet rich in fat Raper and Smith also find that the amount of fat in the liver declines during dosage with insulin, while the fat content in the muscles increases. In opposition hereto stand the findings made by the present author. Although Raper and Smith employed the same method for producing fatty liver as that adopted in this work, yet no comparison can be drawn between their findings and those of the author, owing to the important differences in the arrangement of the experiments, especially the circumstance that Raper and Smith operated with decerebrated cats in which the hypophysis had been destroyed. The rôle played by the hypophysis in fat metabolism has as yet been little investigated, but we know from clinical experiences as well as from experiments on animals that its importance in this respect is very great.

The influence of insulin on fatty livers, whether produced by a diet rich in fat or arising as sequel to pancreas diabetes or phlorizin diabetes, varies, as is seen, very greatly. The fat accumulation in the liver due to pancreas or phlorizin diabetes diminishes after administration of insulin, whereas the fatty liver produced in rabbits by feeding with fat is found in the experiments reported rather to increase its content of fat under the influence of insulin. This difference in the effect of insulin must, however, be regarded in connection with the divergency that has also been observed in the action of insulin on the carbohydrate metabolism in these different subjects for experiment.

The effects of insulin on the fat metabolism observed by the different authors in animals with n o r m a l content of fat in the liver are, as we see, highly divergent. The causes hereof may also be manifold. The plan of procedure varies in the different experiments and the chemical methods employed by the individual investigators for fat determination are also dissimilar. On the one hand, all methods can hardly be said to be equally reliable for fat determination in samples of organs and, on the other hand, the fat components which are being determined by the different methods differ from each other. When to this is added the fact that also the size of the doses of insulin employed in the different experiments varies considerably, we need not be surprised to find that the results of the experiments

may be somewhat divergent. Owing to the mode of procedure which Vendég adopted the results of his experiments are particularly interesting. His experiments, which, it is true, were carried out on normally fed animals, also show that the content of fat in the liver increases during administration of insulin. Vendég interprets the increase as being due to formation of fat from carbohydrates.

From the two experiments which the present author performed on normally fed rabbits no far-reaching conclusions can be drawn with respect to the action of insulin on the fat content of the organs. These two experiments were carried out as a supplement to the experiments on animals with livers rich in fat and may here serve for purposes of comparison, since the mode of procedure and the chemical methods are otherwise alike.

b. *The Action of Insulin on the Glycogen Content in Liver and Muscles.*

The effects produced by insulin on the content of glycogen in liver and muscles have been the object of investigation in numerous experiments during the years that have elapsed since insulin was first discovered. Nevertheless it cannot be said that the manner in which insulin acts on the glycogen is known, the results of the experimental work being far too divergent. One thing certain, however, is that the effect of insulin on the glycogen in the cell is not always one and the same, seeing that the cell reacts sometimes with formation, sometimes with break-down of glycogen. In the pancreas-diabetic organism the administration of insulin will bring about a deposition of glycogen in the liver. This takes place both when food is given together with the insulin and when the animals are allowed to fast (CORI (32)). Whether or not the administration of insulin increases the content of glycogen in the muscles of pancreas-diabetic animals is more doubtful. The glycogen content in the muscles of pancreatectomised animals does not decrease so rapidly as the glycogen in the liver. CHAIKOFF (25) found in fasting dogs with pancreas diabetes a glycogen content of the usual dimension 5 days after administration of insulin ceased. In four dogs the content of glycogen (the average for four muscles) varied between 0.47 and 0.23 per cent. DEBOIS (43), however, found in pancreas-diabetic dogs that the power of building up glycogen in the muscles, when the content thereof has fallen owing to exhaustion of the muscle, is dependent on the administration of insulin.

In phlorizinised animals also the content of glycogen in the liver increased during treatment with insulin (MÖBIUS, WERTHEIMER, CORI (32, 105, 140, 141)).

In fasting animals the effect of insulin on liver and muscle glycogen has been found to vary. FRANK, NOTHMANN and HARTMANN (53) operated with half-grown rabbits which had fasted for 4 days before the experiments

began, whereby the animals came to have a low and comparatively uniform content of glycogen, not exceeding 0.3 per cent in the liver. On administration of small doses of insulin the glycogen content in the liver might now, in short experiments lasting about 4 hours, be found to increase up to 3.8 per cent. The muscle glycogen showed no change. In a dog which had fasted for 5 days it was also found possible in an experiment lasting 6 hours to show an increase of glycogen from 0.4 to 1.02 per cent. In this case a large dose of insulin was given, about 20 clinical units. GOLDBLATT (62—64) worked with young rabbits weighing from 300 to 400 g, which he starved for 24 hours before the experiments. These animals had likewise a very low content of glycogen in the liver. After small doses of insulin he found a considerable increase in liver glycogen, while at the same time, however, a decrease of muscle glycogen took place in several experiments. But Goldblatt thinks that this fall in muscle glycogen is not sufficient to explain the increase in the content of glycogen in the liver. Goldblatt supposes that the increase of glycogen in the liver may have arisen through gluco-neogenesis and that the insulin hypoglycæmia stimulates this new-formation of carbohydrates. BARBOUR, CHAIKOFF, MACLEOD and ORR (6) allowed rats to starve for 48 hours and then gave them small doses of insulin, the animals being killed 1, 1½ and 2 hours afterwards. The results of these experiments show that after the smallest dose of insulin, 1 unit per kg, there comes after the first hour a decrease of liver glycogen, which, again, 2 hours later is altered to an increase. The muscle glycogen decreases under the influence of insulin. POGÁNY (115) made a determination of the total content of glycogen in the whole organism after administration of insulin and found that after small doses (0.5 to 1 clinical unit per kg) the combined amount of glycogen increased. Even though in these experiments a loss of glycogen from the muscles has occurred simultaneously with an increase in the liver, yet the insulin is in these cases found to have led to a net increase of glycogen in the organism. When Pogany gave his mice such large doses of insulin that hypoglycæmic convulsion set in, the glycogen rapidly disappeared from the organism. NIELSEN (107) investigated the effects of insulin on surviving perfused rabbit livers and found that doses larger than ⅛ I. U. led to a loss of liver glycogen, which found expression in an increase of the sugar content in the perfusion liquid. If smaller doses than ⅛ I. U. were given the content of sugar in the liquid decreased during the process of perfusion in the same manner as in experiments without adminstration of insulin. In all these experiments the percentage of sugar in the perfusion liquid lay considerably above the normal blood sugar level. On injection of rather large doses of insulin into fasting normal animals CORI (32) found either no change in the amount of liver glycogen or else a decrease thereof. CORKILL (41) made similar findings in an Australian plant-eating animal (Trichosurus). BÜRGER and KOHL (23) injected insulin intraportally into dogs in somnifen narcosis and determined

the glycogen content of the liver at different times during the experiment. Without administration of insulin there took place in the course of the experiment a steady fall of glycogen in the liver, while if insulin was given intraportally (0.4 to 0.6 units of crystalline insulin per kg) the fall in liver glycogen was slightly more rapid than in the control experiments. CORI and CORI (36) found that administration of insulin (0.75 units per 100 g of weight) to normal rats in postabsorptive state accelerates the decomposition of glycogen in the liver, while the muscle glycogen does not show a greater decrease in the insulin experiments than in the control tests.

From the examples given above of action of insulin on the liver glycogen under various experimental circumstances it appears that it is in animals whose livers are beforehand very poorly supplied with glycogen that insulin brings about an increase in liver glycogen. The livers of pancreas-diabetic and phlorizin-diabetic animals, for example, are very poor in glycogen and in such cases dosage with insulin leads to deposition of glycogen in the liver. Goldblatt and Frank and his co-workers employed young animals and starved them for so long before the experiment that the content of glycogen in the liver was very low (0.2 to 0.3 per cent) and in these animals an increase of glycogen in the liver was observed after administration of small doses of insulin.

In the other experiments it is found that after very small doses of insulin an increase in the glycogen content may take place, whereas insulin administered in large doses was either without effect or else led to decomposition of glycogen. In the experiments which have been carried out in the present work and in which large doses of insulin were employed it cannot be decided with certainty whether the insulin has been without influence on the decomposition of glycogen in the liver or whether it has brought about an increased glycogenolysis. Meanwhile the experiments have shown that when examining the question of changes in the liver's content of glycogen we cannot omit to investigate at the same time the behaviour of the muscle glycogen. From the above-cited works of other authors respecting the effects of insulin on the glycogen in the organism it is also seen that the muscle glycogen often decreases somewhat under the influence of insulin. Corkill maintained that this effect of insulin is in reality an action of adrenalin which has been brought about through an increased secretion of adrenalin in the suprarenals as a result of the dosage with insulin. The same view has also been adopted by Cori. In the investigations described in this work there has also been observed in most of the insulin experiments a decrease of the content of glycogen in the muscles and the most natural manner of explaining this decrease is to assume, as does Corkill, that it is due to an action of adrenalin. The glycogen in the muscles is broken down to lactic acid by the adrenalin. As lactic acid can be converted into glycogen in the liver, it is possible that the break down of glycogen which in many of the insulin experiments was seen to take place in the muscles has

led to formation of glycogen in the liver. It may therefore be imagined that the fall in liver glycogen observed during the experiments with insulin would have been greater if the muscle glycogen had not been attacked at the same time. A similar view has been advanced as regards the control experiments. Also in the most of these experiments the muscle glycogen showed a fall at the same time as the liver glycogen decreased.

c. *Summary.*

If the effects of insulin on the glycogen content and on the fat content of the liver are regarded in combination, it will be seen from the above-cited experiments of other authors that the experiments in which the quantity of fat in the liver decreases during administration of insulin are also those in which its content of glycogen increases. Both in pancreatic and in phlorizinic diabetes the liver has a large content of fat and a very scanty supply of glycogen. After administration of insulin the liver cells will augment their content of glycogen, while at the same time the store of fat will diminish.

In livers in which the accumulation of fat is due to other factors than a pancreatic or a phlorizinic diabetes we do not, however, find the same effect from the insulin. The fatty liver due to poisoning by phosphorus it was not possible to remedy by administration of insulin, but neither was it possible by means of insulin to increase the content of glycogen in the liver.

The fatty liver produced by feeding with high fat diet differs from the diabetic livers and from the fatty liver due to phosphorus in the fact that the glycogen content is not greatly reduced. As is seen from the tables, the author has found in these fatty livers produced by diet a glycogen content of more than 5 per cent even after from 18 to 24 hours' fasting. In many cases, it is true, a considerably lower content of glycogen was recorded, down to as low as 0.3 per cent, but even in these cases the poverty in glycogen cannot be regarded as very pronounced. Whereas the accumulation of fat in the fatty liver due to diabetes or phlorizin, and doubtless also in that caused by phosphorus poisoning, is of secondary nature, the storage of fat in the cells of the fatty livers produced by feeding is a primary process evoked by the lively metabolism of fat. The glycogen content in such livers has no significance for the occurrence of fatty liver of this type. This discovery made it at once less probable that one could bring about a reduction of the fat content in the fatty liver due to diet through an increase of the glycogen content in the organ.

The experiments made with administration of insulin to fat-fed animals have shown that the content of glycogen in the liver is reduced thereby and the fat content increased. These findings, however, do not prove, as might at first glance be supposed, that under these conditions there never-

theless exists an antagonism between glycogen and fat in the liver. For in the control experiments there also occurs a decline in the liver's content of glycogen, without its being possible to detect any material increase in the percentage of fat. The rise in the fat content in the liver seems therefore to be directly brought about by administration of insulin. No increase of the glycogen content has at any time been found in these experiments, nor has there ever been observed any reduction in the quantity of fat.

One manner in which this accumulation of fat in the liver during dosage with insulin might be explained is to assume that a migration of fat to the liver has taken place. In several of the insulin experiments the increase of fat in the liver was accompanied by a reduction of the quantity of fat in the muscles. It is possible that there is a correlation between this decrease of fat in the muscles and the increase thereof in the liver, but otherwise it might be imagined that it is the fat tissue that has supplied material for the accumulation of fat in the liver. It may be objected that the changes occurring in the blood's content of fat during these insulin experiments are very small and do not seem to stand in proportion to the increased transportation of fat that must have taken place. Meanwhile the fat content in the blood is dependent, on the one hand, on the rapidity with which the fat is mobilised and conveyed to the blood vessels and, on the other hand, on the celerity with which the fat passes from the blood into the organs in which it is to be stored. Provided the peripheral mobilisation of the fat and its deposition in the liver keep pace with each other, no great changes in the fat content of the blood need necessarily occur. The tendency to a decrease in the quantity of fat in the blood which has nevertheless been observed in these experiments would seem to indicate that the rapidity with which the hepatic cells have taken up fat from the blood has actually exceeded the speed with which the fat is mobilised in other parts of the organism. Cf. LEATHES and RAPER (85, p. 151). A migration of fat from the depots to the liver in case of fatty liver due to administration of other hormones has been demonstrated. BARRET, BEST and RIDOUT (7), using a method of labelling fatty acids with deuterium, thus found that the fat wich accumulates in the liver when certain extracts of the anterior pituitary gland are administered is derived from the body depots.

The possibility also exists that the fat deposited in the liver has been formed therein from glucose. GEELMUYDEN (58, p. 96), together with other authors, maintains that insulin promotes the formation of fat from sugar. The loss of carbohydrates which, as may be seen from the results, has occurred during the experiments is, however, hardly sufficient alone to explain the accumulation of fat in the liver, if it is a fact that the formation of fat from sugar proceeds in the proportions which BLEIBTREU (13) and MAGNUS-LEVY (97) supposed.

Mann and Magath's experiments in liver extirpation have shown that dehepatised animals must constantly be given glucose in order to maintain

the normal blood sugar content. Assuming that the blood sugar in normal animals is, at any rate partly, maintained by a new-formation of sugar (from protein and possibly fat), it might be supposed that also this sugar became again converted to fat under the influence of the insulin. In order to account for the increase of fat in the liver in such case it would, of course, be necessary to assume that the fat which formed the initial material in this reversible process lay outside the liver.

VII. Experiments with Insulin + Glucose and with Glucose Alone.

1. Liver Fat in Relation to Liver Glycogen. Experimental Results with Brief Discussion.

In the preceding chapter the view was maintained that the changes which took place in the fat content of the liver during intravenous infusion of a thin solution of insulin in 0.9 per cent NaCl into rabbits fed with fat did not stand in any causal relationship to the alterations occurring at the same time in the content of glycogen in the liver. It may be objected that the experiences on which this view is based are not sufficiently complete, seeing that the glycogen in the liver decreased during all the experiments with insulin and in no single case was an increase of glycogen observed simultaneously with a rise in the fat content of the liver. It might therefore be found desirable to supplement the insulin experiments by some new experiments where variations in the fat content in the liver could be studied during simultaneous increase in the amount of glycogen in the organ. A number of experiments were carried out on fat-fed rabbits to which insulin was administered together with large quantities of sugar. As likewise this mode of procedure led to no essential increase in the content of glycogen in the liver, some experiments were carried out with administration of glucose alone. In these different experiments the glucose was conveyed either to the duodenum or direct into the blood vessels, while the insulin was given intravenously. In the cases where the glucose was introduced into the duodenum, it was done through a laparotomy incision, a 40 per cent solution of glucose being injected by means of a syringe into the upper part of the intestine. The disadvantage of this procedure is that one does not know how much of the glucose is absorbed during the period of experiment. In one of the experiments glucose was still present in the coecum at the end of the period, 5 hours after the glucose had been injected into the duodenum. The results of these experiments are set forth in tabular form (Tables 16, a—d) in the same manner as in the case of the earlier experiments. The results of two experiments with insulin + glucose and of two with glucose alone are also presented graphically (Figs. 18—21).

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HYPOGLYCEMIA ACTIVATES COMPENSATORY MECHANISM OF GLUCOSE METABOLISM OF BRAIN

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The effect of plasma glucose concentration on the cerebral uptake of [^{18}F]-fluorodeoxy-D-glucose (FDG) was studied in a broad concentration range in a rabbit brain model using dynamic FDG PET measurements. Hypoglycemic and hyperglycemic conditions were maintained by manipulating plasma glucose applying i.v. glucose or insulin load. FDG utilization (K) and cerebral glucose metabolic rate (CGMR) were evaluated in a plasma glucose concentration range between 0.5 mM and 26 mM from the kinetic constant k_1, k_2, k_3 obtained by the Sokoloff model of FDG accumulation. A decreasing set of standard FDG uptake values found with increasing blood glucose concentration was explained by competition between the plasma glucose and the radiopharmacon FDG. A similar trend was observed for the forward kinetic constants k_1, and k_3 in the entire concentration range studied. The same decreasing tendency of k_2 was of a smaller magnitude and was reverted at the lowest glucose concentrations where a pronounced decrease of this backward transport rate constant was detected. Our kinetic data indicate a modulation of the kinetics of carbohydrate metabolism by the blood glucose concentration and report on a special mechanism compensating for the low glucose supply under conditions of extremely low blood glucose level.

Keywords: Dynamic FDG PET – CGMR – hypoglycemia

INTRODUCTION

Glucose is a major substrate for brain energy metabolism, and a significant fraction of glucose metabolism is coupled to neuronal activity. It is of critical importance for normal function and resistance to injury in both the mature and developing brain [1, 21]. The plasma membranes of most mammalian cells contain one or more proteins, which transport glucose by facilitated diffusion. The principal glucose transporters in the brain are GLUT1 (present at high concentration at the blood brain barrier and astrocytes), GLUT3 isoforms expressed in neurones and GLUT5 in microglia [15, 16, 28].

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Although brain glucose metabolism is basically phosphorylation limited [17] transport processes may become rate limiting under special conditions. Changes in the expression of GLUT 1 and GLUT 3 have been reported during postnatal development [19, 26], following transient focal ischemic injury [25] and under other special conditions [4, 15]. Great attention has been given to the changes in the D-glucose transport activity in hyperglycemic or hypoglycemic state [5, 11, 12, 13, 17, 19, 20, 29], but this intensively studied issue still has a number of unsolved questions and it has resulted in controversial results as well. A substantial part of the related investigations has been based on detecting changes in protein and/or RNA synthesis or transporter protein degradation, but research on the putative glucose dependent regulation of the brain glucose metabolism is required.

PET, using [^{18}F]-2-fluoro-2-deoxy-D-glucose (FDG), enables the *in vivo* measurement of regional glucose metabolism under physiological and pathological conditions alike. FDG utilisation can be quantitized by using compartmental analysis [22] allowing the processes comprising the glucose metabolism to be investigated in detail. In this study, we sum up about the relationship between the kinetic parameters of FDG accumulation in rabbit brain and the plasma glucose level in a broad concentration range using dynamic PET investigations. Data analysis has revealed a special regulatory mechanism activated by extremely low blood sugar concentrations.

MATERIALS AND METHODS

Animals

Rabbits of 2.7 ± 0.5 kg (mean ± SD, n = 17) were used throughout the experiments. The animals were provided *ad libitum* access to food except for the last five-hour fasting period prior to the PET investigations. The experimental protocol was approved by the Laboratory Animal Care and Use Committee of University Medical School of Debrecen based on the Helsinki declaration. In every experimental group (control, hyper- and hypoglycemic group) at least 5 animals were used. The rabbits were anaesthetised with i.p. urethane (0.5 g/kg) and alpha-chloralose (50 mg/kg). One femoral artery and one femoral vein were cannulated [7]. Glucose loading (40% glucose) and insulin (Actrapid MC 40 IU/ml, NOVO) was applied i.v. to manipulate the plasma glucose concentration. Hyperglycemia was achieved applying a glucose load of 2–3 ml 40% glucose/kg body weight, 120 minutes prior to the FDG PET study, followed by an another 5 ml dose 90 minutes later. Hypoglycemia was induced applying 15–20 IU insulin (120 minutes prior to the PET scan) followed by another insulin administration (10 IU), 30 minutes prior to the FGD injection. Plasma glucose levels were measured before the glucose and insulin treatments and during the PET scans as indicated in Fig. 1. Two animals were twice subjected to dynamic PET investigations under different conditions. Rabbit No 1 was manipulated in the first experiment in a way to have 26 mM, the highest (in the whole set of measurements), blood glucose concentration and two days later, the same animal was investigated

under extremely low blood glucose concentration (1.1 mM glucose). Rabbit No 2 was adjusted first to hypoglycemic condition (2 mM blood glucose concentration) and two days later this animal was used as a control with 7.3 mM blood glucose concentration.

Serial arterial blood sampling was performed in all cases to allow the kinetic analysis of FDG accumulation.

PET scanning

FDG was synthesised according to Hamacher et al. [6]. The radiochemical purity of ^{18}FDG was higher than 97%, and the specific activity was close to 2 Ci/μmol. PET scans were carried out using a GE 4096 whole body PET camera. The laboratory animals were intravenously given 2–4 mCi FDG in 2 ml physiologic salt solution as a 20–30 sec bolus. All PET measurements were of dynamic type with the number and length of the exposures as follows: 12×30 sec, 5×1 min, 5×3 min, and 3×5 min. Data used for the construction of static images were collected between the 40th and the 60th min. Transmission scans obtained with a Ge^{68}/Ga^{68} external rotating rod source have been used to correct for tissue attenuation.

The regional FDG uptake was expressed as the standardized uptake value (SUV) and was calculated as the ratio of the decay-corrected tissue concentration (in mCi/kg) and the injected dose per body weight (in mCi/kg).

Dynamic analysis of brain glucose metabolism was carried out using the three compartment Sokoloff's model of glucose accumulation [22] as modified by Phelps et al. [18] including the dephosphorylation rate constant of FDG. Kinetic rate constants (k_1 is the rate constants of the glucose transport from the intravasal compartment to the intracellular compartment, k_2 is the rate constant of the reverse transport, k_3 and k_4 are the rate constants of the phosphorylation and dephosphorylation reaction, respectively) of the model were evaluated using the matrix representation of the model [8]. The rate of FDG utilization (K, expressed in 1/min units) and the cerebral glucose metabolic rate ($CGMR$ expressed in mg/100 g/min) were calculated as:

$$K = \frac{k_1 k_3}{k_2 + k_3}$$

$$CGMR = \frac{C_p}{Lc} \frac{k_1 k_3}{k_2 + k_3}$$

where C_p denotes plasma glucose concentration (mg/100 g), Lc is the Lumped constant, [22] the numerical value of which was assumed to be 1.

RESULTS

The plasma glucose concentrations of the untreated animals (without glucose and insulin load) were in the range of 5–12 mM (7.86 ± 1.5, n = 6). Administration of glucose brought about an immediate increase in the blood glucose concentration followed by a slower decrease. Applying a second glucose load we succeeded to maintain a relatively constant hyperglycemic level of glucose during the PET scan (Fig. 1). The manipulated plasma glucose level of this group of animals ranged from 13 to 26 mM (20.7 ± 5.3, n = 6). Our insulin administration protocol resulted in an abrupt decrease of the plasma glucose concentration prior to the PET scan (Fig. 1B) and was efficient in maintaining very fairly stable hypoglycemic glucose levels in the range between 0.5 and 2.6 mM (1.66 ± 0.84, n = 5) during the PET measurements. For a

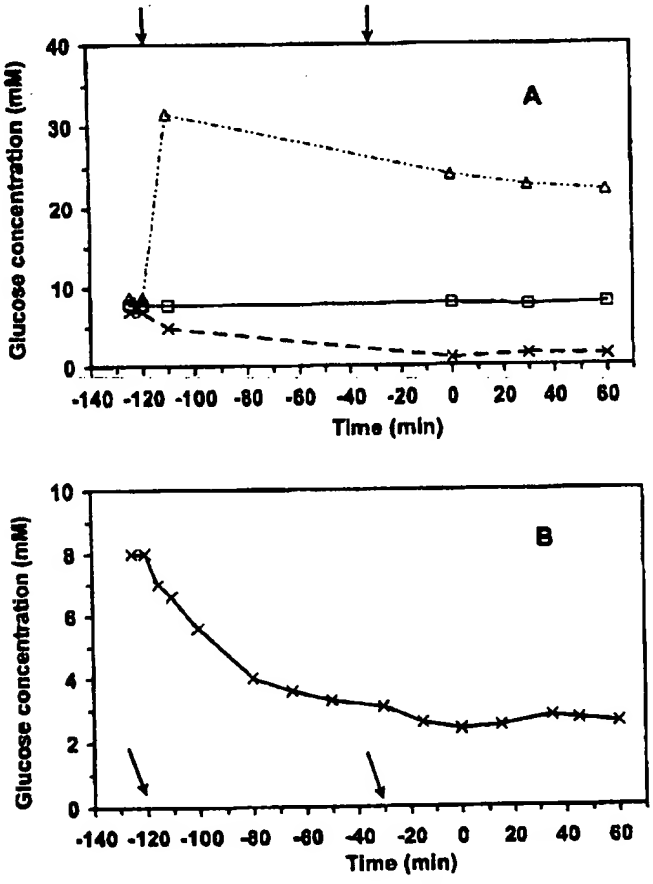

Fig. 1. **A:** Characteristic time dependence of plasma glucose concentration in a representative euglycemic (□), hypoglycemic (×) and hyperglycemic (△) animal. **B:** Time course of the plasma glucose concentration upon insulin treatment in a single experiment. Hypoglycemia was induced by i.v. administration of 20 IU insulin 120 minutes prior to the FGD injection followed by another 10 IU insulin dose 90 minutes later. Arrows indicate time points of glucose and insulin administration

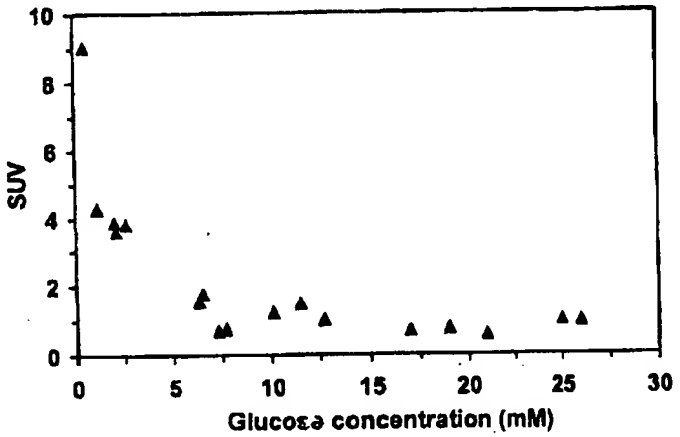

Fig. 2. Effect of plasma glucose concentration on the standard uptake value of FDG accumulation in rabbit brain

representative experiment a mean plasma glucose concentration of 2.6 ± 0.15 mM was obtained from data displayed in Fig. 1B averaged over the time of emission data collection.

The SUV were determined for the whole brain and plotted against plasma glucose concentration. The results displayed in Fig. 2 show a pronounced decrease of FDG accumulation with rising concentration of the glucose in the blood.

Results of the kinetic analysis are shown in Figs 3–5. Kinetic constant k_1 shows a monotonous decrease with growing blood glucose concentration in the whole range investigated (Fig. 3). Below 2 mM plasma glucose level, the numerical value of this

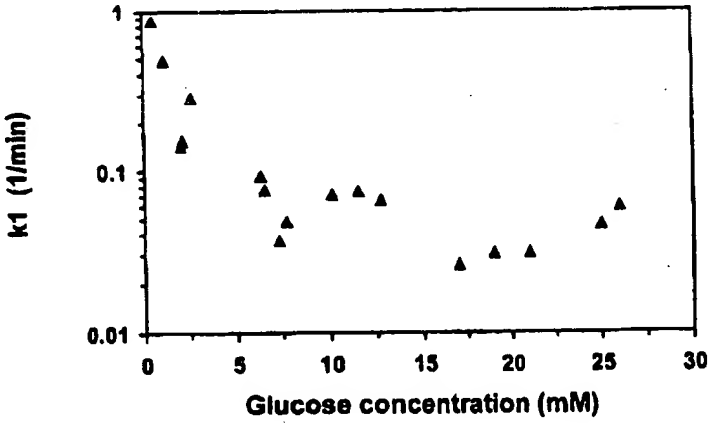

Fig. 3. Effect of plasma glucose concentration on the k_1 kinetic constant of glucose metabolism in the rabbit brain

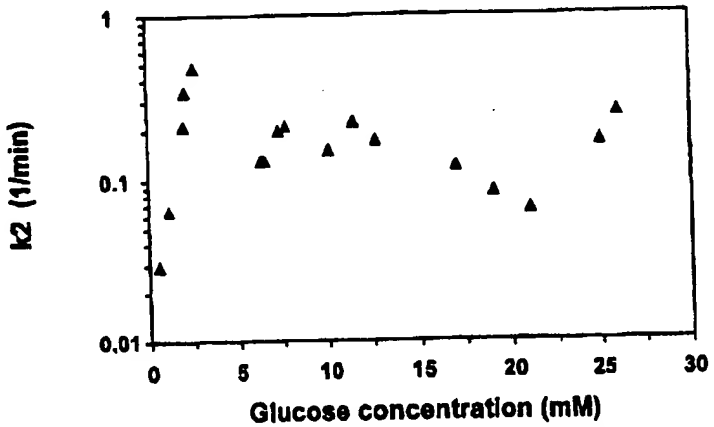

Fig. 4. Effect of plasma glucose concentration on the k_2 kinetic constant of glucose metabolism in the rabbit brain

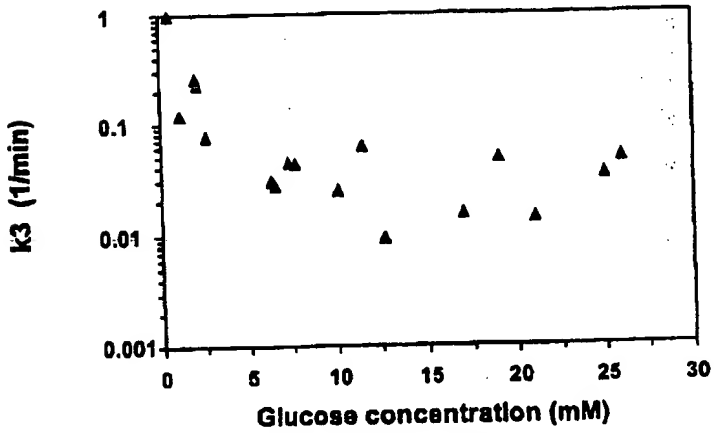

Fig. 5. Effect of plasma glucose concentration on the k_3 kinetic constant of glucose metabolism in the rabbit brain

constant increases by a factor of 8. A similar tendency can also be observed in the change of k_3 (Fig. 5). The concentration dependence of these forward parameters is very similar to each other under euglycemic and hyperglycemic conditions. In contrast, although only a slight change was detected in k_2, characterizing the inverse transport of FDG from the intracellular compartment to the intravasal space, in the hyperglycemic and normoglycemic range, a remarkable decrease in its numerical value was detected at low plasma glucose levels (Fig. 4). At very low plasma glucose concentration (0.5–1 mM) parameter k_2 approached zero which was accompanied by a 5–10 times increase in the values of k_1 and k_3. The kinetic constants of the hexokinase catalysed glucose phosphorylation decreased monotonously with increasing plasma glucose levels.

Known values of k_1, k_2 and k_3 obtained from kinetic model calculations allowed the evaluation of the rate of FDG utilisation (Fig. 6) and the rate of cerebral glucose metabolism (Fig. 7). The former parameter showed a steadily decreasing tendency with the increase of the blood glucose level. Interestingly, the calculated CGMR vs plasma glucose concentration displayed minimum value in the physiological blood glucose concentration range and increased in both the hyperglycemic and hypoglycemic direction.

Two animals were reused in the experiments under conditions of different plasma glucose concentrations. With rabbit No. 1 hyperglycemic (extremely high level) con-

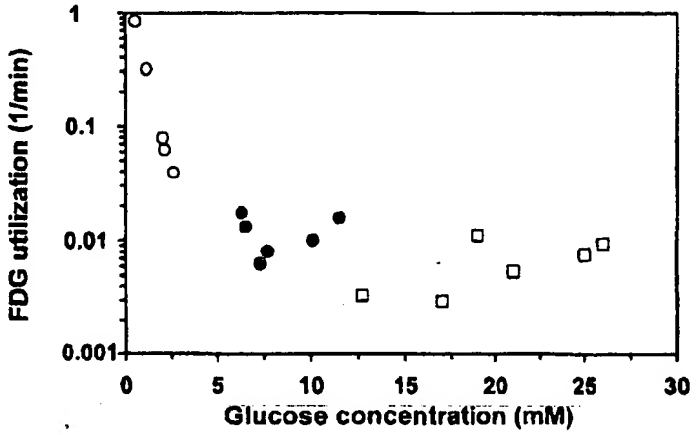

Fig. 6. Effect of plasma glucose concentration of the FDG utilization rate in the rabbit brain (euglycemic [(●), hypoglycemic (○) and hyperglycemic (□)]

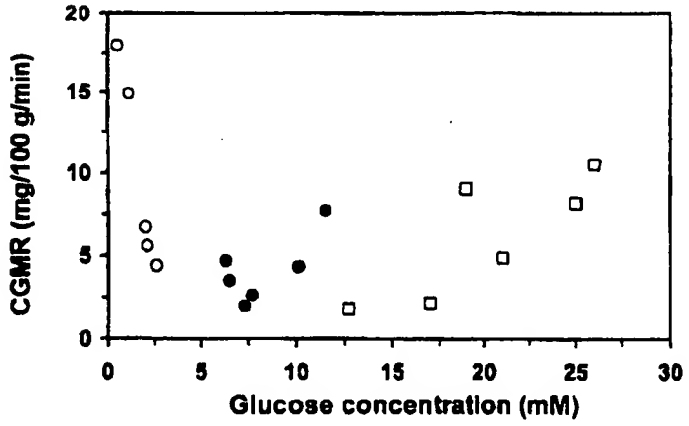

Fig. 7. Effect of plasma glucose concentration on the glucose metabolic rate (CGMR) in the rabbit brain [euglycemic (●), hypoglycemic (○) and hyperglycemic (□)]

ditions were maintained first and in the experiment two days later FDG PET scan was completed on the same animal with hypoglycemic plasma glucose level. Rabbit No. 2 was subjected to the first measurement under hypoglycemic conditions and served as a control with physiological blood glucose concentration two days later in a second experiment. The results of the kinetic analyses of these measurements are summarised in Table 1. The results obtained with these rabbits fitted in well with the trend of other measurements in rabbits used in only a single run.

Table 1

Kinetic parameters of brain glucose metabolism on the rabbits each used under two different conditions of plasma glucose concentration

	Glucose, mM	K_1, l/min	k_1, l/min	k_2, l/min	k_3, l/min	LGMR, mg/100 g·min
Rabbit 1	26.0	0.00944	0.05944	0.25513	0.04817	10.5
Rabbit 1	1.1	0.31600	0.48809	0.06545	0.12063	14.9
Rabbit 2	2.0	0.07869	0.14270	0.21387	0.26280	6.74
Rabbit 2	7.3	0.00639	0.03650	0.19870	0.04406	2

DISCUSSION

Having special intention to carry out dynamic investigations of glucose metabolism in the widest possible range of glucose concentration, we have chosen a laboratory animal experimental system. The rabbit brain has been selected as an experimental system for this purpose as the rabbit model has proved to be suitable for PET investigations [3, 10]. It has also been suggested that the evaluation of the kinetic constants is superior to the analysis of the FDG utilization rate as the former can reflect metabolic changes more specifically. The precise calculation of glucose metabolic rate assumes exact knowledge about the numerical value of the Lumped constant. As no *Lc* data are available on rabbit brain we have postulated $Lc = 1$. We assumed also that the value of the Lumped constant for rabbit brain does not change with the blood glucose concentration. This assumption has been made in analogy with the finding by Suda et al. [23] and Dienel et al. [2] who reported relatively stable Lumped constant for the rat brain in a wide range of plasma glucose concentration. Fairly stable normoglycemic and hypoglycemic plasma glucose concentrations were maintained during the time range of the PET measurements with the protocol outlined (Fig. 1). Manipulation of the blood glucose was carried out during the last 2 hours before the dynamic PET scans. This time interval proved to be long enough for the regulatory mechanisms to produce alterations in the kinetic parameters and it could also be compared with the reported ones during which the changes in glucose transporter expression (or translocation) and glucose transporter mRNA levels develop [29].

FDG uptake in rabbit brain tissue was inhibited by increased glucose concentration in the blood as it was seen from the inverse relationship between SUV values and plasma glucose level (Fig. 2). This finding was consistent with the competition between FDG and glucose. The extent of competition and the displacement of FDG by plasma glucose were determined by the K_m values for these substrate molecules [22]. The relatively low K_m values of GLUT1 and GLUT3 transporters resulted in a decline of the FDG uptake by the brain as the plasma glucose levels rose [17]. Moreover, FDG is a substrate analogue for hexokinase, thus both substrates compete for the binding/active site of this enzyme as well. Due to the competition of different level and other factors, the effect of blood glucose concentration on the FDG accumulation may be tissue dependent. Accordingly, the FDG uptake of different tissues was inhibited to different extent by high plasma glucose levels [9, 14, 24, 27, 28]. Glucose dependent changes in the individual rate constants of the FDG accumulation were studied in detail. Kinetic constant k_1, determining the transport rate from the intravasal space toward the extracellular and (non-phosphorylated) intracellular space, decreased monotonously with increasing glucose levels. Change in k_1 over the 5–26 mM glucose concentration range was surpassed by that over the 1–5 mM range, the latter amounting to about an order of magnitude (Fig. 3). Glucose transport toward the tissues can become rate limiting at very low plasma glucose level and this may activate defined compensatory regulation mechanisms to increase the rate of this transport process.

Of interest is the finding that change in the reverse transport rate parallels that of k_1 only above 3 mM glucose concentration but, below this threshold, an abrupt decrease of k_2 takes place. A similar change in the values of k_1 and k_2 would be consistent with a simple, glucose concentration induced change, also including the possibility of altered expression of the appropriate glucose transporter molecules or a translocation of these proteins. The change of opposite sign in these parameters means that these regulatory processes alone, even if they exist, cannot account for the detected behaviour of the transport constants. Allowing for a direct competition or an altered expression or even a translocation process, we still have to assume additional factors exerting different effect on forward and backward transport. One of the possibilities can be the appearance of a metabolite of a regulatory concentration in the hypoglycemic tissue environment, binding of which may result in altered transport characteristics of the transporter protein molecules but this assumption requires further experimentation.

The glucose concentration dependence of the FDG utilization rate is displayed in Fig. 6 as calculated from the numerical values of k_1, k_2 and k_3. This derived parameter shows the largest dynamic range with varying glucose concentration as it has contribution from all the three individual rate constants. It is interesting to note that the slight individual increase in the k_1, k_2 and k_3 parameters at the highest glucose levels results in a pronounced increase of the CGMR in this concentration range (Fig. 7). The rising trend of CGMR at the lowest blood glucose levels tested reports on an overcompensation of the loss in CGMR expected at very low blood glucose level by changing the molecular parameters of the involved elementary processes.

In conclusion this study demonstrates that FDG uptake decreases in rabbit brain in animals with elevated blood glucose concentration. The reduced SUV values are in accord with the diminished numerical values of k_1, and k_3 in the studied blood glucose concentration range. Kinetic constant k_2 changes in a very similar way except in the lowest, 1 mM–3 mM, concentration range. The observed changes are explained by a direct competition between FDG and glucose as well as an additional special compensatory mechanism.

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